Applicants : Seetharama A. Acharya and Belur N. Manjula

Appl. No. : Not Yet Assigned (National Stage of PCT/US2003/040407)

Filed : Herewith

Page 3

## Amendment to the Claims:

A listing of the claims, including Claims 7, 17-19 and 22 as currently amended, is presented below.

- 1. (Original) A hemoglobin molecule (Hb) having six  $\pm$  one PEG chains, wherein two of said PEG chains are bound to Cys-93( $\Re$ ) of Hb, and the remaining PEG chains are bound to thiol groups introduced on  $\Re$ -NH<sub>2</sub> of Hb.
- 2. (Original) The hemoglobin molecule of Claim 1, wherein each PEG chain has a molecular weight of 3000-10,000 daltons.
- 3. (Original) The hemoglobin molecule of Claim 1, wherein each PEG chain has a molecular weight of about 5000 daltons.
- 4. (Original) The hemoglobin molecule of Claim 1, wherein each PEG chain is linked to Hb by a succinmidyl linkage.
- 5. (Original) The hemoglobin molecule of Claim 2, wherein each PEG chain is linked to Hb by a succinmidyl linkage.
- 6. (Original) The hemoglobin molecule of Claim 3, wherein each PEG chain is linked to Hb by a succinmidyl linkage.
- 7. (Currently Amended) The hemoglobin molecule of any of Claim 1 Claims 1-6, which is non-hypertensive.

Applicants

Seetharama A. Acharya and Belur N. Manjula

Appl. No.

Not Yet Assigned (National Stage of PCT/US2003/040407)

Filed

Herewith

Page 4

- 8. (Original) A process for preparing a modified hemoglobin molecule (Hb), comprising the steps of:
  - (a) reacting Hb with 8-15 fold excess of iminothiolane to form thiolated Hb; and
  - (b) reacting the thiolated Hb with 16-30 fold excess of PEG functionalized with a maleimide moiety, to form the modified Hb.
- 9. (Original) The process of Claim 8, wherein Hb is reacted with 9-12 fold excess iminothiolane in step (a).
- 10. (Original) The process of Claim 8, wherein Hb is reacted with about 10 fold excess iminothiolane in step (a).
- 11. (Original) The process of Claim 8, wherein the thiolated Hb is reacted with 18-22 fold excess PEG functionalized with a maleimide moiety in step (b).
- 12. (Original) The process of Claim 8, wherein the thiolated Hb is reacted with about 20 fold excess PEG functionalized with a maleimide moiety in step (b).
- 13. (Original) The process of Claim 8, wherein Hb is reacted with 9-12 fold excess iminothiolane in step (a), and the thiolated Hb is reacted with 18-22 fold excess PEG functionalized with a maleimide moiety in step (b).
- 14. (Original) The process of Claim 8, wherein Hb is reacted with 9-12 fold excess iminothiolane in step (a), and the thiolated Hb is reacted with about 20 fold excess PEG functionalized with a maleimide moiety in step (b).

Applicants

:

Seetharama A. Acharya and Belur N. Manjula

Appl. No.

Not Yet Assigned (National Stage of PCT/US2003/040407)

Filed

Herewith

Page 5

- 15. (Original) The process of Claim 8, wherein Hb is reacted with about 10 fold excess iminothiolane in step (a), and the thiolated Hb is reacted with 18-22 fold excess PEG functionalized with a maleimide moiety in step (b).
- 16. (Original) The process of Claim 8, wherein Hb is reacted with about 10 fold excess iminothiolane in step (a), and the thiolated Hb is reacted with about 20 fold excess PEG functionalized with a maleimide moiety in step (b).
- 17. (Currently Amended) The process of any of Claim 8 Claims 8-16, wherein PEG has a molecular weight of 3000-10,000 daltons.
- 18. (Currently Amended) The process of any of Claim 8 Claims 8-16, wherein PEG has a molecular weight of about 5,000 daltons.
- 19. (Currently Amended) The process of <del>any of Claim 8 Claims 8-16</del>, wherein the modified Hb is non-hypertensive.
- 20. (Original) The process of Claim 17, wherein the modified Hb is non-hypertensive.
- 21. (Original) The process of Claim 18, wherein the modified Hb is non-hypertensive.
- 22. (Currently Amended) A modified Hb prepared by the process of any of Claim 8 Claims 8-16.
  - 23. (Original) A modified Hb prepared by the process of Claim 17.

**Applicants** 

:

Seetharama A. Acharya and Belur N. Manjula

Appl. No.

Not Yet Assigned (National Stage of PCT/US2003/040407)

Filed

Herewith

Page 6

24. (Original) A modified Hb prepared by the process of Claim 18.

25. (Original) A modified Hb prepared by the process of Claim 19.

26. (Original) A modified Hb prepared by the process of Claim 20.

27. (Original) A modified Hb prepared by the process of Claim 21.